Chapter III

Materials and Methods

Instruments

1. Spectrophotometer - Pye Unicam SP 1800, and recorder
2. pH meter - Radiometer Copenhagen, PHM 62 STANDARD pH METER
3. Temperature controlled - water bath - Memmert

Chemicals

1. Dicyclomine hydrochloride U.S.P. (99.46 % purity on dried basis as determined by USP method[1])
2. Bromcresol green (BCG) - Fluka A.G.
3. Methyl yellow - Fluka A.G.
4. Chloroform - Baker Analyzed
5. Sodium hydroxide - Baker Analyzed
6. Sodium lauryl sulfate - Fluka A.G.
7. Sodium sulfate anhydrous - BDH
8. Potassium hydrogen phthalate - May and Baker
9. Potassium dihydrogen phosphate - May and Baker
10. Potassium chloride - BDH
11. Hydrochloric acid - Riedel - De
12. Sulfuric acid - Riedel - De

All of the chemicals were analytical grade obtained from various manufacturers. Dicyclomine hydrochloride was pharmaceutical grade.

Reagents

1. Bromcresol green solutions (for Method 1)

   3×10^{-4} M BCG - BCG powder 52.4 mg was dissolved in 250 ml chloroform.

   4×10^{-4} M BCG - BCG powder 69.8 mg was dissolved in 250 ml chloroform.

   1×10^{-3} M BCG - BCG powder 174.5 mg was dissolved in 250 ml chloroform.

2. Bromcresol green solutions (for Method 2)

   3×10^{-4} M BCG - BCG powder 52.4 mg was dissolved in 1.5 ml of 0.1 N sodium hydroxide and diluted with distilled water to 250 ml.

   4×10^{-4} M BCG - BCG powder 69.8 mg was dissolved in 2 ml of 0.1 N sodium hydroxide and diluted with distilled water to 250 ml.

   1×10^{-3} M BCG - BCG powder 174.5 mg was dissolved in 5 ml of 0.1 N sodium hydroxide and diluted with distilled water to 250 ml.

3. Sodium hydroxide solution of various strengths were prepared as followed.

   1 N sodium hydroxide - Sodium hydroxide 9 gm was dissolved in 190 ml distilled water.

   2 N Sodium hydroxide - Sodium hydroxide 18 gm was dissolved in 190 ml distilled water.

   3 N Sodium hydroxide - Sodium hydroxide 27 gm was dissolved in 190 ml distilled water.

4 N Sodium hydroxide - Sodium hydroxide 36 gm was dissolved in 190 ml distilled water.

6 N Sodium hydroxide - Sodium hydroxide 54 gm was dissolved in 190 ml distilled water.

4. Buffer solutions were prepared according to directions in the USP(1).

4.1 Buffer solutions between pH 1.0 and 2.0 were prepared by taking 50 ml of 0.2 M potassium chloride solution, adding 134.0 and 13.0 ml of 0.2 M hydrochloric acid solution respectively, and then adding enough distilled water to make 200 ml.

4.2 Buffer solutions between pH 3.0 and 5.0 were prepared by taking 50 ml of 0.2 M potassium biphthalate solution, adding 22.3 and 0.1 ml of 0.2 M hydrochloric acid solution, and 22.6 ml of 0.2 M sodium hydroxide solution respectively, and then adding enough distilled water to make 200 ml.

4.3 Buffer solutions between pH 6.0 and 8.0 were prepared by taking 50 ml of monobasic potassium phosphate solution, adding 5.6, 29.1 and 46.1 ml of 0.2 M sodium hydroxide solution respectively, and then adding enough distilled water to make 200 ml.

4.4 Buffer solution pH 9.0 was prepared by taking 50 ml of 0.2 M boric acid and potassium chloride solution, adding 20.8 ml of 0.2 M. sodium hydroxide solution, and then adding enough distilled water to make 200 ml.

5. Methyl yellow TS - A stock solution of methyl yellow in alcohol was diluted to obtain a solution having a concentration of 0.10 mg/ml

6. 0.004 M sodium lauryl sulfate - Sodium lauryl sulfate 1.15 g was dissolved in about 500 ml of water, 2 ml of sulfuric acid was added, and diluted with water to 1000 ml., and mixed.

7. 2 N Sulfuric acid

8. Chloroform

Standard Solutions

1. Method 1 - Dicyclomine HCl 172.9 mg was dissolved in 50 ml of distilled water and mixed well to produce a concentration of 3.5 mg/ml.

2. Method 2 - Dicyclomine HCl 69.8 mg was dissolved in 100 ml of chloroform. This stock solution was diluted to give the concentration of 1×10^{-4} M and 2×10^{-4} M working standard, which were prepared freshly for each run.

Methods

1. Determination of Maximum Absorption Wavelength

Method 1

A 10.0 ml quantity of dicyclomine hydrochloride standard solution, 3.5 mg/ml, was pipetted into a 125 - ml separator. Twenty five ml of 2 N sodium hydroxide was then added and the solution was extracted with two 25 ml portions and three 10 ml portions of chloroform. Each chloroform addition was shaken for 1 minute and allowed to stand for 5 minutes. Each chloroform extract was filtered through cotton overlaid with anhydrous sodium sulfate and collected in a 100-ml volumetric flask. The combined chloroform extracts was adjusted to

volume with chloroform and diluted successively with chloroform to get approximate 1 x 10^{-4} M (Solution A) and 2 x 10^{-4} M (Solution B) of dicyclomine standard solution.

A 5.0 ml quantity of Solution A was pipetted into a 25-ml volumetric flask, 5 ml of 4 x 10^{-4} M BCG was added and the resulting solution was diluted to volume with chloroform. The maximum absorption wavelength of the complex formed was determined spectrophotometrically by scanning in a 1-cm cell against a reagent blank in the visible range from 350 - 550 nm. The procedure was repeated three times and the absorption spectrum obtained was shown in Figure 1.

Method 2

A 5.0 ml quantity of 1 x 10^{-4} M dicyclomine hydrochloride standard solution was pipetted into a 125-ml separator. A 5 ml chloroform, 5 ml 4 x 10^{-4} M BCG and 25 ml buffer pH 2 were added. The solution was shaken for 1 minute and allowed to stand for 2 minutes. The chloroform layer was filtered through cotton overlaid with anhydrous sodium sulfate into a 25-ml volumetric flask and the filter was rinsed with a few ml of chloroform. The aqueous layer was extracted again with two further 5 ml portions of chloroform and the filtered chloroform extracts were collected in a 25-ml volumetric flask. The resulting solution was diluted to volume with chloroform. The maximum absorption wavelength was determined as the same in Method 1 and the result obtained was also shown in Figure 1.

2. Determination of the Appropriate Normality of Sodium Hydroxide Solution Used in Liberating Dicyclomine Free Base from Dicyclomine Hydrochloride and the Completion of Extraction

Ten ml quantities of Dicyclomine hydrochloride standard solution, 3.5 mg/ml , were pipetted into five 125-ml separators. Twenty five ml of 1 N, 2 N, 3 N, 4 N and 6 N sodium hydroxide solution were then added respectively. Twenty five ml chloroform was added, and the solution was shaken vigorously for 1 minute, and allowed to stand for 5 minutes. The chloroform layer was filtered through cotton overlaid with anhydrous sodium sulfate into 100-ml volumetric flask. The extraction was repeated again with one 25.0 ml and two further 10.0 ml portions of chloroform. The funnel was rinsed down with chloroform. The aqueous phase was continue extracted with a 10.0 ml chloroform and the filtrate (the fifth chloroform extract) was collected in a 25-ml volumetric flask. The same procedure was reapeated again with another 10.0 ml chloroform and the filtrate (the sixth chloroform extract) was collected in another 25-ml volumetric flask. Five ml quantities of 4×10^{-4} M BCG were then added to the fifth and sixth chloroform extract of each solution, and the resulting solutions were diluted to volume with chloroform. The absorbance of each solution was measured against a reagent blank at 415 nm. The procedure was repeated three times and the results obtained were shown in Table 1.

3. Determination of the Effect of pH

Five ml quantities of 1 x 10^{-4} M dicyclomine hydrochloride standard solution were pipetted into nine 125-ml separators. Five ml chloroform and 5 ml 4 x 10^{-4} M BCG were added. Then 25 ml quantities of buffer solution pH 1-9 were added to the separators respectively. Each separator was shaken for 1 minute and allowed to stand for 2 minutes. Then each chloroform extract was filtered through cotton overlaid with anhydrous sodium sulfate into a 25-ml volumetric flask and the filter was rinsed with a few ml of chloroform. The procedure was repeated again with two further 5 ml portions of chloroform. The chloroform extracts were combined in a 25-ml volumetric flask and the solution was diluted to volume with chloroform. The reagent blank of each pH was prepared as the same condition described above but without dicyclomine hydrochloride. The absorbance of the complex formed of each pH was measured at 415 nm. against a reagent blank. The procedure was repeated four times and the results obtained were shown in Table 2 and Figure 2.

4. Determination of the Effect of Time on Stability of Dicyclomine - Bromcresol Green Complex

Method 1

A 5.0 ml quantity of Solution A (prepared as directed under Method 1 "Determination of Maximum Absorption Wavelength".) was pipetted into a 25-ml volumetric flask. Then 5 ml of 4×10^{-4} M BCG was added and the resulting solution was diluted to volume with chloroform. The absorbance of the complex formed was measured at 415 nm at 0, 15, 30, 60, 120, 180, 240, 300 and 360 minutes against a reagent blank.

Method 2

A 5.0 ml quantity of 1×10^{-4} M dicyclomine hydrochloride standard solution was pipetted into a 125-ml separator. Then 5 ml chloroform, 5 ml 4×10^{-4} M BCG and 25 ml buffer pH 2 were added. The separator was shaken for 1 minute and allowed to stand for 2 minutes. The chloroform extract was filtered through cotton everlaid with anhydrous sodium sulfate into a 25-ml volumetric flask and the filter was rinsed with a few ml of chloroform. The procedure was repeated again with two further 5 ml portions of chloroform. The chloroform extracts were combined in a 25-ml volumetric flask and the resulting solution was diluted to volume with chloroform. The absorbance of the extracted complex was measured at 415 nm at 0, 15, 30, 60, 120, 180, 240, 300 and 360 minutes against a reagent blank.

Both methods were repeated three times and the results obtained were shown in Table 3 and Figure 3.

5. Determination of the Effect of Temperature on Stability of Dicyclomine - Bromcresol Green Complex

Method 1

Five ml quantities of Solution A (prepared as directed under Method 1 "Determination of Maximum Absorption Wavelength") were pipetted into four 25-ml volumetric flasks. Then 5 ml of 4×10^{-4} M BCG and 10 ml chloroform were added to each flask. The solutions were heated in a water - bath at 40, 50, 60 and 70°C for 15 minutes. The solutions were then cooled to room temperature and diluted to volume with chloroform. The absorbances of the complex were measured at 415 nm against a reagent blank. The results were compared with that obtained at room temperature (25°C).

Method 2

Five ml quantities of 1×10^{-4} M dicyclomine hydrochloride standard solution were pipetted into four 125-ml separators. Then 5 ml chloroform, 5 ml 4×10^{-4} M BCG and 25 ml buffer pH 2 were added to each separator. The separators were shaken for 1 minute and allowed to stand for 2 minutes. Each chloroform extract was filtered through cotton overlaid with anhydrous sodium sulfate into a 25-ml volumetric flask and the filter was rinsed with a few ml. of chloroform. The procedure was repeated again with two further 5 ml portions of chloroform. The chloroform extracts were combined in a 25-ml volumetric flask. The solutions were heated in a water - bath at 40, 50, 60 and 70°C for 15 minutes. The solutions were then cooled to room temperature and diluted to volume with chloroform. The absorbances of the complex

were measured at 415 nm. against a reagent blank. The results were compared with that obtained at room temperature (25 ^{o}C).

Both methods were repeated three times and the results obtained were shown in Table 4 and Figure 4.

6. Determination of Maximum Dye Concentration

Method 1

Three ml. quantities of Solution B (prepared as directed under Method 1 "Determination of Maximum Absorption Wavelength") were pipetted into eight 25-ml volumetric flasks. Then several aliquots of 3×10^{-4} M BCG : 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0 and 8.0 ml were added respectively and all of the solutions were diluted to volume with chloroform. Reagent blanks were prepared as directed above but without dicyclomine. The absorbances of the complex obtained were measured at 415 nm against reagent blanks.

Method 2

Three ml quantities of 2×10^{-4} M dicyclomine hydrochloride standard solution were pipetted into eight 125-ml reparators. Then several aliquots of 3×10^{-4} M BCG : 0.5, 1.0, 2.0, 3.0, 4.0, 6.0 and 8.0 ml were added respectively. Twenty five ml quantities of buffer pH 2 were added, and the resulting solutions were shaken for 1 minute and allowed to stand for 2 minutes. Then each chloroform extract was filtered through cotton overlaid with anhydrous sodium sulfate into a 25 ml volumetric flask and the filter was rinsed with a few ml of chloroform. The procedure was repeated again with two further 5 ml portion of chloroform. Each chloroform extract was collected in a 25-ml volumetric flask and the resulting solution was diluted to volume with chloroform. Reagent blanks were prepared as directed above but without dicyclomine. The absorbances of the complex obtained were measured at 415 nm againt reagent blanks.

Both methods were repeated four times. Curves were obtained by plotting between milliliters of 3×10^{-4} M BCG and absorbance readings. The results were shown in Table 5 and Figure 5. The mole ratio curves were also obtained by plotting between mole ratio of dye to drug and absorbance readings; and the results were shown in Figure 6.

7. Determination of Adherence to Beer's Law

Method 1

Several aliquots of Solution B (prepared as directed under Method 1 "Determination of Maximum Absorption Wavelength") : 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, and 6.0 ml were pipetted into eight 25-ml volumetric flasks. Then 3 ml quantities of 1×10^{-3} M BCG were added respectively and the resulting solution were diluted to volume with chloroform. The absorbance of each solution was measured at 415 nm against a reagent blank.

Method 2

Several aliquots of 2×10^{-4} M dicyclomine hydrochloride standard solution : 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, and 6.0 ml were pipetted into eight 125-ml separators and each solution was diluted to 10 ml with chloroform. Then, to each solution, 3 ml 1×10^{-3} M BCG and 25 ml buffer pH 2 were added. The separators were shaken for 1 minute and allowed to stand for 2 minutes. Each chloroform extract was filtered through cotton overlaid with anhydrous sodium sulfate into a 25 ml volumetric flask and the filter was rinsed with a few ml of chloroform. The procedure was repeated again with two further 5 ml portions of chloroform. Each chloroform extract was collected in a 25-ml volumetric flask and the resulting solution was diluted to volume with chloroform. The absorbance of each solution was measured at 415 nm against a reagent blank.

The procedure in both methods were repeated five times and calibration curves were obtained by plotting between absorbance

readings and concentrations of dicyclomine hydrochloride. The results obtained were shown in Table 6 and Figure 7.

8. Determination of the Percent Labelled Amount of Dicyclomine HCl in Dicyclomine HCl Tablet by Using Method 1 and USP Method[1]

Method 1

Twenty dicyclomine tablets (10 mg/tab.) were weighed and finely powdered. A portion of the sample mixture equivalent to about 5 mg of dicyclomine hydrochloride was accurately weighed and transfered into a 125-ml separator containing 10 ml of distilled water, and the separator was swirled for 5 minutes. Then 25 ml 2 N sodium hydroxide was added and the aqueous phase was extracted with two 25 ml and three 10 ml portions of chloroform. Each chloroform addition was shaken for 1 minute and allowed to stand for 5 minutes. Then each chloroform extract was filtered through cotton overlaid with anhydrous sodium sulfate into a 100-ml volumetric flask and the resulting solution was diluted to volume with chloroform. A 4.0 ml quantity of chloroform solution was pipetted into a 25-ml volumetric flask, 5 ml 4×10^{-4} M BCG was added and the resulting solution was diluted to volume with chloroform. The absorbance of the complex formed was measured at 415 nm against a reagent blank. The amount of dicyclomine hydrochloride in tablet was calculated from the following formula :

$$\% \text{ dicyclomine hydrochloride} = \frac{Au \times Cs \times D \times Wt. \times 100}{As \times W \times P}$$

Au = Absorbance of the unknown

As = Absorbance of the standard

Cs = Concentration of standard

D = Dilution factor

P = Label amount

W = Weight of sample

Wt = Average weight per tablet

USP Method

Twenty dicyclomine tablets (10 mg/tab.) were weighed and finely powdered. A portion of the sample mixture equivalent to about 20 mg of dicyclomine hydrochloride was accurately weighed and transfered to a glass - stoppered 100-ml cylinder. Then 20 ml water, 5 ml 2 N sulfuric acid, 20 ml chloroform, and 1 ml of methyl yellow TS were added respectively. Insert the stopper in the cylinder and the solution was shaken well until a bright yellow color was produced in the chloroform phase. The solution was titrated with 0.004 M sodium lauryl sulfate, and shaked throughly after each addition, until the first permanent orange - pink color was produced in the chloroform phase. Each ml of 0.004 M sodium lauryl sulfate is equivalent to 1.384 mg of dicyclomine hydrochloride. The amount of dicyclomine hydrochloride in tablets was calculated from the following formula : -

$$\% \text{ dicyclomine hydrochloride} = \frac{V \times N \times 1.384 \times Wt \times 100}{0.004 \times W \times P}$$

V = Volume of sodium lauryl sulfate solution

N = Normality of sodium lauryl sulfate solution

W = Weight of sample

Wt = Average weight per tablet

P = Label amount

Both methods were repeated ten times and the results obtained were shown in Table 8.

9. Determination of the Percent Recovery of Dicyclomine HCl in Dicyclomine HCl Tablet by Method 1 and USP Method

Method 1

Several aliquots of the sample mixture (which known amount of dicyclomine hydrochloride from the pervious determination) equivalent to about 3 mg of dicyclomine hydrochloride were accurately weighed into three 125-ml separators containing 10 ml distilled water. Then 2.0, 3.0 and 4.0 ml of dicyclomine hydrochloride standard solution, 1 mg/ml in distilled water, were added into the separators respectively. All of the solutions were swirled for 5 minute and the same proce - dure was proceeded as described under determination of the percent labelled amount of dicyclomine hydrochloride in dicyclomine hydro- chloride tablet.

The percent recovery was calculated from the following formula:-

$$\% \text{ recovery of dicyclomine hydrochloride} = \frac{(Wf - Ws)}{Wa} \times 100$$

Wf = Weight of dicyclomine hydrochloride found

Ws = Weight of dicyclomine hydrochloride from tablet

Wa = Weight of dicyclomine hydrochloride added

USP Method

Several aliquots of the sample mixture (which known amount of dicyclomine hydrochloride from the previous determination) equivalent to about 18 mg of dicyclomine hydrochloride were accurately weight and transfered to glass - stoppered 100 ml cyclinders. Then 2.0, 3.0 and 4.0 ml of dicyclomine hydrochloride standard solution, 1 mg/ml in

distilled water, were added to the cylinder respectively. The rest of the procedure was the same as described under determination of the percent labelled amount of dicyclomine hydrochloride in dicyclomine hydrochloride tablet, beginning with the word "Then 20 ml of water, 5 ml 2 N sulfuric acid, 20 ml chloroform, and _ _ _ _ _ _ _". The percent recovery was calculated as described under Method 1.

The procedure was repeated five times for each method and the results obtained were shown and compared in Table 9.

10. Comparative Analysis of Preparations Containing Dicyclomine HCl

To test the validity of the method, five commercially available formulation with different dosage forms were analyzed by Method 1 compared with USP method.

Method 1

The accurately measured amounts of tablet (capsule or syrup) equivalent to about 5 mg of dicyclomine hydrochloride was transfered into a 125-ml separator containing 10 ml of distilled water and the procedure described under determination of the percent labelled amount of dicyclomine hydrochloride in dicyclomine hydrochloride tablet. The procedure was repeated five times and the results obtained were shown and compared with USP method in Table 10.

USP Method

1. Tablet and Capsule

The same procedure was carried on as described under determination of percent labelled amount of dicyclomine hydrochloride in dicyclomine hydrochloride tablet.

2. Syrup

An accurately measured volume of syrup, equivalent to about 20 mg of dicyclomine hydrochloride was transfered into a 125-ml separator containing 10 ml of water and 1 ml of hydrochloric acid. The solution was extracted with three 20-ml portions of chloroform and the chloroform extracts were collected in a glass- stoppered 100-ml cylinder. Then 5 ml of diluted sulfuric acid, 1 ml methyl yellow and 5 ml water

were added. The procedure was proceeded as directed in determination of the percent labelled amount of dicyclomine hydrochloride in dicyclomine hydrochloride tablet. The results obtained were shown in Table 10.